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Variants of wheat histone H1 with N- and C-terminal extensions

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Two histone H1 variants have been isolated from wheat germ with M_r in the region 23000–27000. Partial sequences from their globular regions are reported.

Histone H1 variant *(Wheat embryo)* M, *Amino acid sequence*

1. INTRODUCTION

The occurrence of histone variants is well established [1]. The expression of such species-specific variants during particular stages of differentiation has been described for sea urchin embryos [2–4] as well as chicken embryo [5]. The widespread species- and differentiation stage-specific occurrence of isohistones in the animal kingdom suggests that histone variants may be significant for the modelling of specific chromatin structures. The structural information on plant histones and their variants is less well developed. We have characterized by complete and partial sequencing several structural variants of the H2A [6] and H2B group [1] from wheat embryos.

Gel electrophoresis suggested a number of isohistones of the H1 and H2B class in wheat germ chromatin [6,7]. To identify unequivocally structural variants it is necessary to demonstrate sequence differences. However, the close similarity of the isohistones and the abundance of polysaccharides make preparative isolation in good yields often difficult, thus preventing elucidation of the complete structures. Therefore, in this investigation we have used microsequencing of homologous fragments prepared from presumptive isohistones of the H1 subgroup, to prove the existence of structural histone H1 variants.

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2. MATERIALS AND METHODS

Wheat germ histones were extracted and fractionated on Biogel P60 as described [6]. The histone H1 fraction thus isolated was further fractionated by ion-exchange chromatography (see fig.1). Polyacrylamide gel electrophoresis was performed in acid-urea gels (2.5 M urea) [8], in SDS at pH 8.8 [9] and in Triton X-100 in 6 M urea [10]. M_r values of the proteins and some of their fragments were determined electrophoretically in acidic urea gels as described [11].

Amino acid compositions were determined after 24 h hydrolysis in 5.7 N HCl. No correction factors for incomplete hydrolysis or destruction of amino acids were applied. Histones H1 were fragmented with CNBr as in [4] and the peptides separated by gel filtration. Fragment $CN1_{(3)}$ was purified on a Waters μBondapak C-18 reverse-phase column with a linear acetonitrile gradient in 0.05 N trifluoroacetic acid. Sequential Edman degradation was performed in the 0.5–10 nmol range using a modified gas-phase sequenator [12]; Pth-amino acids were analysed by reverse-phase chromatography on Lichrosorb RP 8 as described [13]. All amino acid composition data and amino

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acid sequences of CNBr fragments are based on multiple analyses.

3. RESULTS AND DISCUSSION

Wheat histones separated on size-exclusion chromatography into the core histone fractions and histone H1 [6]. This H1 fraction appears heterogeneous in the absence and presence of detergent (SDS) on electrophoresis and moves with an M_r considerably larger than that of calf thymus histone H1 [6]. The presumptive H1 fraction resolves on trisacryl ion-exchange chromatography into 3 main fractions (fig.1); the leading and trailing component correspond on gel electrophoresis to the fastest and slowest moving subfraction. The subfraction with intermediate electrophoretic mobility could not be purified by rechromatography on amberlite or carboxymethylcellulose using methodology described previously [13–15]. The amino acid composition of the fractions thus separated is typical for H1 type histones (table 1). However, the electrophoretic mobility in SDS suggests a larger M_r than that hitherto considered typical for histones H1 [6,7].

The M_r of the wheat histones can be more reliably computed from their mobility in acid-urea gels, and the charge-to-mass ratio determined by amino acid analysis [11]. The thus established M_r values of the 3 wheat histones H1 are in the range 23000–27000 (table 1), i.e. these histones contain between 230 and 270 amino acid residues. This makes them considerably larger than somatic animal histones H1 which possess approx. 200 residues. We have reported previously that the histones H2A and H2B from wheat also exhibit larger M_r values [1,6].

The H1 subfractions $H1_{(1)}$ and $H1_{(3)}$ have blocked N-termini. On CNBr cleavage $H1_{(1)}$ and

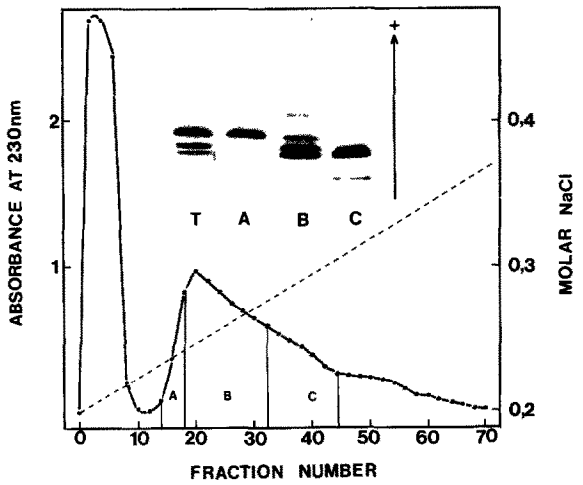

Fig.1. Ion-exchange chromatography of wheat histone H1. The H1 histones were isolated as described [6] and dissolved in 6 M urea-1% mercaptoethanol and applied to a 0.9 × 10 cm CM-Trisacryl column using 0.05 M Tris-acetate (pH 5.3) buffer and a linear NaCl gradient to elute the proteins. Fractions were collected and pooled as indicated. SDS-polyacrylamide gels of a typical run of the total histone (T) and the 3 major fractions (1–3) are shown in the inset. Proteolysis of H1 during the purification has been minimized by using freshly extracted protein solutions and by the presence of $PhMeSO_3F$ (0.0001 M) in all solutions used for the isolation and purification.

Table 1

Amino acid composition of wheat germ histone H1 subfractions (fig.1) (mol%)

Amino acid	CM-Tris-acryl fraction			Total H1 P-60[a]	Calf thymus H1
	1	2	3		
Lys	24.4	24.6	25.0	22.1	28.5
His	1.1	1.2	1.3	1.5	–
Arg	2.2	2.7	2.7	3.0	1.7
Asp	2.3	2.8	2.5	3.1	2.2
Thr	5.3	5.8	6.7	5.5	5.9
Ser	3.7	4.3	3.8	4.6	6.5
Gly	3.4	3.5	3.0	4.8	3.8
Pro	11.7	13.8	12.7	11.5	8.8
Gly	2.2	2.5	2.2	3.6	6.9
Ala	32.2	29.7	29.9	27.4	25.0
Val	4.7	4.1	3.3	4.7	4.8
Met	0.4	0.3	0.8	0.7	0.0
Ile	2.3	1.5	1.5	2.0	0.7
Leu	2.9	2.5	2.3	3.2	4.3
Tyr	0.9	0.4	0.8	1.2	0.4
Phe	0.8	0.4	0.5	1.1	0.5
N-terminal	blocked	blocked	blocked	–	blocked

[a] P-60 fraction 1 was obtained after molecular exclusion chromatography of total wheat histones on Biogel P-60 using 0.02 N HCl as eluant [6]. The electrophoretic patterns of all fractions are shown in fig.1

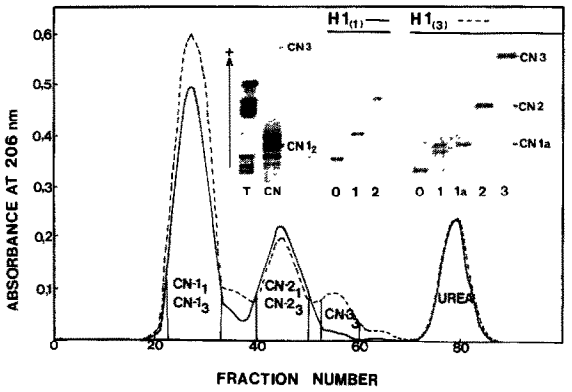

Fig.2. Separation of peptides generated from wheat embryo histones H1 after CNBr cleavage. Wheat histones $H1_{(1)}$ (——) and $H1_{(3)}$ (– – –) corresponding to CM-Trisacryl fractions 1 and 3 (fig.1) were subjected to CNBr cleavage followed by size-exclusion chromatography on a 150 × 1.5 cm Sephadex G-75 column using 0.02 N HCl as eluant. (Inset) SDS-polyacrylamide gels of the various CNBr fractions. T, total wheat histones; CN, unfractionated CNBr fragments of total H1 (P-60 fraction 3) [6]; $H1_{(1)}$:0, uncleaved histone $H1_{(1)}$ (fraction 1, fig.1); 1, CN-1; 2, CN-2. $H1_{(3)}$:0, uncleaved histone $H1_{(3)}$; 1, CN-1; 1a, CN-1 purified by reverse-phase chromatography; 2, CN-2; 3, CN-3. Electrophoresis and sequence analysis reveal that fraction CN-1 from $H1_{(3)}$ is heterogeneous containing 2 unblocked fragments. One of the sequences in fraction CN-1 $H1_{(3)}$ was identical to that of CN-3. Therefore the larger fragment in CN-1 must be the product of either an incomplete cleavage or a closely related variant missing the second methionine residue.

Table 2

Amino acid composition of CNBr fragments of CM-Tris-acryl fraction 1 (histone $H1_{(1)}$) and fraction 3 (histone $H1_{(3)}$ (fig.1) (mol%, figures in brackets are the most likely number of residues per molecule, derived from the M_r estimates and the best integer)

Amino acid	Histone $H1_{(1)}$				Histone $H1_{(3)}$				
	CN1	CN2	CN1+2	$H1_{(1)}$	CN1[a]	CN2	CN3	CN1+2+3	$H1_{(3)}$
Lys	26.9 (48)	14.1 (7)	(55)	24.4 (56)	29.6 (53)	16.0 (8)	14.4 (6)	(67)	25.0 (68)
His	1.0 (2)	1.6 (1)	(3)	1.1 (3)	0.8 (1)	2.8 (1)	6.7 (2)	(4)	1.3 (4)
Arg	1.9 (4)	2.0 (1)	(5)	2.2 (5)	2.4 (4)	1.9 (1)	4.3 (2)	(7)	2.7 (7)
Asp	1.4 (3)	5.0 (3)	(6)	2.3 (5)	1.1 (2)	4.9 (3)	5.8 (2)	(7)	2.5 (7)
Thr	5.4 (10)	6.8 (3)	(13)	5.3 (12)	6.5 (12)	8.7 (4)	4.4 (2)	(18)	6.7 (18)
Ser	3.4 (7)	4.2 (2)	(9)	3.7 (9)	3.2 (6)	4.3 (2)	5.1 (2)	(10)	3.8 (10)
Glu	2.5 (5)	6.2 (3)	(8)	3.4 (8)	1.4 (3)	5.7 (3)	7.5 (3)	(9)	3.0 (8)
Pro	12.5 (22)	14.8 (7)	(29)	11.7 (27)	16.0 (29)	13.6 (7)	5.5 (2)	(38)	12.7 (34)
Gly	2.6 (5)	2.4 (1)	(6)	2.2 (5)	2.2 (4)	1.8 (1)	5.2 (2)	(7)	2.2 (6)
Ala	31.4 (56)	29.3 (15)	(71)	32.2 (74)	30.5 (55)	30.0 (15)	21.2 (8)	(78)	29.9 (81)
Val	3.8 (7)	8.2 (4)	(11)	4.7 (11)	2.3 (4)	3.6 (2)	4.4 (2)	(8)	3.3 (9)
Met	0.0[a] (0)	1.0[b] (1)	(1)	0.4 (1)	0.0[b] (0)	T[b] (1)	T[b] (1)	(2)	0.8 (2)
Ile	2.3 (4)	1.4 (1)	(5)	2.3 (5)	0.9 (2)	0.7 (0)	6.2 (2)	(4)	1.5 (4)
Leu	3.6 (6)	0.9 (1)	(7)	2.9 (7)	2.5 (4)	0.3 (0)	4.4 (2)	(6)	2.3 (6)
Tyr	0.5 (1)	1.1 (1)	(2)	0.9 (2)	0.4 (1)	1.6 (1)	0.6 (0)	(2)	0.8 (2)
Phe	0.8 (2)	0.0 (0)	(2)	0.8 (2)	0.2 (0)	0.1 (0)	4.0 (2)	(2)	0.5 (1)
Total residues	(182)	(51)	(233)	(232)	(180)	(49)	(40)	(269)	(267)
N-terminus	Val	blocked		blocked	Leu	blocked	Val		blocked
M_r[c]	19400	6400		24300	19700	7000	4600		27500

[a] This peptide was purified by reverse-phase chromatography (see fig.2)
[b] Apparent from the presence of homoserine
[c] Determined on acid-urea gels after correcting the electrophoretic mobility for differences in the cationic charge [11]

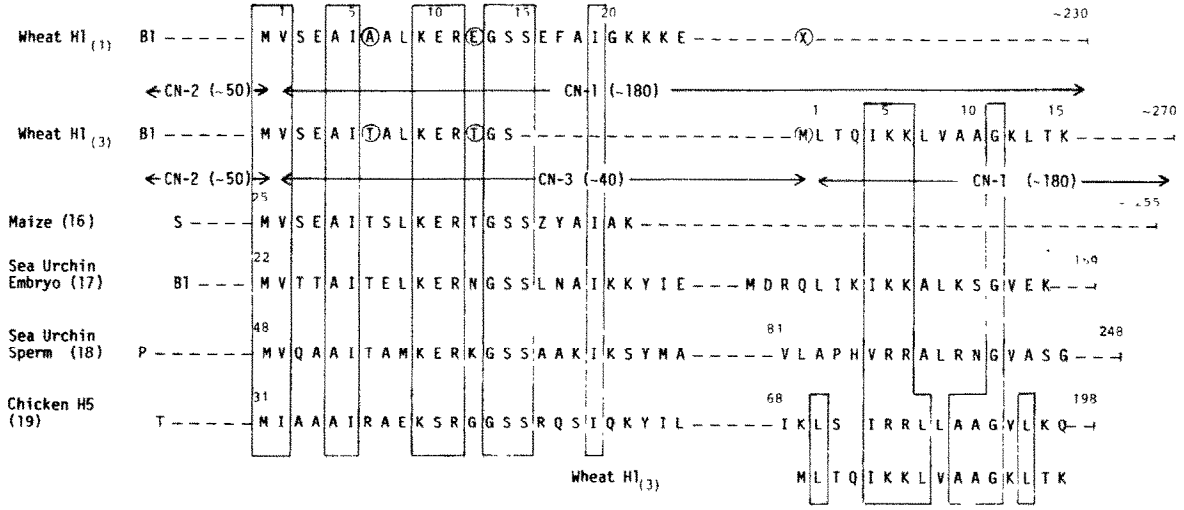

Fig.3. Summary of the structural data for the wheat histones $H1_{(1)}$ and $H1_{(3)}$ and comparison to other H1 histones. Homologous regions have been boxed. The number in brackets indicates the total estimated length of that particular fragment (table 2). Differences in $H1_{(1)}$ and $H1_{(3)}$ have been encircled.

$H1_{(3)}$ yield 2 and 3 fragments, respectively (fig.2), in accordance with their methionine content (table 1). The small CN-2 fragments from $H1_{(1)}$ and $H1_{(3)}$ are blocked and each comprise approx. 50 residues representing the N-terminal region. Fragments CN-1 constitute the C-terminal part of the molecules and contain the alanine-lysine rich regions and are approx. 180–190 residues in length (table 2). The electrophoretic pattern of the CN-1 fragments closely resembles the pattern of the uncleaved molecules (fig.2). Thus, the heterogeneity of the H1 fraction resides mainly in the C-terminal sections of the component histone H1 molecules (fig.2). As expected from the methionine content (table 1), $H1_{(3)}$ yields an additional fragment, CN-3 (fig.2, table 2). Its electrophoretic mobility corresponds to an M_r of 4000, i.e. it comprises approx. 40 residues.

To characterize further these proteins between 0.5 and 8 nmol of the various peptides were subjected to gas-phase sequencing (fig.3, table 3). The rapidly increasing background of lysine and alanine made extended sequencing runs difficult. Although the amino acid compositions of the CN-1 fragments of histones $H1_{(1)}$ and $H1_{(3)}$ are closely related (table 2) they are definitely derived from different proteins, evident from the different post-methionine sequences (table 3). Based on homology to histone H5 and other H1 histones we assume the CN-3 fragment derived from histone $H1_{(3)}$ must have been generated from the centre region of the hydrophobic domain and must be aligned with respect to CN-1 towards the N-terminus of the molecule. $H1_{(1)}$ and $H1_{(3)}$ differ from one another in at least 3 positions in the regions sequenced, i.e. in fragments CN-1 Ala 6 has been replaced by Thr, Glu 12 by Thr and an unknown amino acid by Met.

These results establish that in wheat embryos the histone H1 fraction comprises several variants, the main characteristics of which are their moderately elongated N-terminal and the extensively extended C-terminal regions. Considering the amino acid composition of the fragments and the likelihood of internal reiteration of sequence structures [13], the elucidation of their complete structures will have to await the isolation of their genes. We have previously shown that similar extensions of the polypeptide chains apply to the core histones H2A [6]. Out of the 40 residues positioned in the histones H1, 14 and 17 respectively are different if compared to animal histones H1 even in this most conserved region of the molecules. Maize H1 contains only 5 amino acid substitutions in the same region [16]. Except for histones H3 and H4, all wheat embryo histones are larger than the homologous proteins in the animal kingdom. Whether these differences may cause variations in

Table 3

Yields of Pth-amino acids obtained during the isothiocyanate degradation of H1 peptides

Step no.	$H1_{(1)}$ CN-1		$H1_{(3)}$ CN-3		$H1_{(3)}$ CN-1a	
1	Val	3.5	Val	9	Leu	5.0
2	Ser	2.0	Ser	5	Thr	3.0
3	Glu	2.6	Glu	4	Gln	2.2
4	Ala	2.0	Ala	7	Ile	4.0
5	Ile	2.0	Ile	6	Lys	2.4
6	Ala	1.6	Thr	3	Lys	2.0
7	Ala	2.1	Ala	6	Leu	2.8
8	Leu	1.4	Leu	4	Val	3.1
9	Lys	1.4	Lys	5	Ala	3.3
10	Glu	0.6	Glu	3	Ala	4.5
11	Arg	0.5	Arg	2	Gly	1.0
12	Glu	0.6	Thr	1	Lys	1.7
13	Glu	0.9	Glu	3	Leu	0.2
14	Ser	0.8	Ser	0.5	Thr	0.5
15	Ser	0.6	Ser	0.5	Lys	1.0
16	Glu	0.7				
17	Phe	0.6				
18	Ala	0.6				
19	Ile	0.5				
20	Glu	0.2				
21	Lys	0.4				
22	Lys	0.4				
23	Lys	0.4				
24	Glu	0.3				

[a] Peptide $H1_{(3)}$ CN-1 was purified by reverse-phase chromatography (see fig.2)

Yields of Pth-amino acids have been recorded in nmol representing the recovery of the amino acids after the background concentration had been subtracted

the higher order packing of the nucleosomes in wheat embryo chromatin remains to be seen.

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